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THE CLOTTING OF BLOOD AS SEEN WITH THE ULTRAMICROSCOPE

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Several observers have applied the ultramicroscope to the study of the process of clotting. According to Mayer,[1] whose description of the process is confirmed in the main by Cesana,[2] what occurs is first the appearance of minute granules or particles in what was before (Mayer) an optically empty field. These granules then agglutinate to form rows or threads in which the constituent particles are still visible, or in the case of more massive coagulation, such as occurs in normal clotting, the granules agglutinate into masses from which later fibers are formed and eventually a sponge work or honeycombed structure. In a recent paper by Stübel[3] a very different description is given. This author used the dark field illuminating method (parabaloid or cardioid condenser) and was able to show that, in most cases at least, the fibrin is deposited in the form of definite needles which give rise to a meshwork throughout the liquid. The formation of these needles resembles the process of crystallisation. In bird's blood as a rule, and in frog's blood always, according to Stübel the process differs from that seen in mammalian blood in that the fibrin appears as a network instead of as isolated needles.

At the time that Stübel's paper appeared the author was preparing to follow the process of clotting under the ultramicro-

[1] Mayer: La coagulation du plasma sanguin. Comptes rendus de la société de biologie. 1907, lxiii, 658.

[2] Cesana: Ricerche ultramicroscopiche sulla coagulazione del plasma di sangue. Archivio di Fisiologia. 1908, v, 180.

[3] Stübel: Ultramikroskopiche Studien über Blutgerinnung und Thrombocyten. Pflüger's Archiv. 1914, clvi, 361.

scope with the intention of using pure solutions of fibrinogen and thrombin. The results attained are so decisive, in some points, as to merit a brief description. The apparatus used in the work consisted of the slit ultramicroscope of Siedentopf and Zsigmondy for liquid colloids. Illumination was obtained from sunlight reflected by a heliostat. Most of the observations and all of the photographs were made with the water-immersion objective D and ocular 2. The material used for observation consisted of the oxalated blood-plasma from various animals (man, dog, cat, horse, terrapin) which had been centrifugalized at high speed for half an hour, so as to give a corpuscle-free liquid. This plasma was made to clot, while under observation with the ultramicroscope, by the addition of a suitable amount of an aqueous solution of thrombin. The thrombin was made by a method previously described.[4] Its solutions are water-clear. In addition to the clotting of the oxalated plasmas a great many observations were made on mixtures of fibrinogen solutions and thrombin. In these cases the fibrinogen was prepared from plasma by the successive precipitations with half-saturation of sodium chloride. The last precipitate, after centrifugalizing and washing, was dissolved in a 0.9 per cent solution of sodium chloride to which was added a trace of a dilute solution (0.5 per cent) of sodium bicarbonate. The mixtures of plasma and thrombin or of fibrinogen and thrombin were poured into the funnel attached to the cell (Biltz form of apparatus) as soon as made and then immediately brought into the cell for observation.

RESULTS

When the amount of thrombin is adequate to produce a firm clot within a few minutes the process of clotting proceeds in substantially the same way in all plasmas observed. The end-result is the formation of a meshwork of beautiful needles, which stand out brilliantly in the field as rigid acicular structures. The process of clotting, as Stübel says, proceeds after the manner of crystal formation. There is no indication of the existence of

[4] Howell: Rapid method of preparing thrombin. American Journal of Physiology. 1913, xxxii, 264.

fibers or of a network. The character of the meshwork of needles is shown sufficiently well in the illustrations[5] (figs. 1, 2, 3), so that a further description is unnecessary. The matter of greatest interest was to attempt to follow the actual process of formation of these needles. With the slit-ultramicroscope this is possible up to a certain point. When the proper proportion of thrombin is used the process takes place uniformly throughout the field of observation. Bright specks appear first as short rods, which exhibit a genuine saltatory movement, jumping abruptly into and out of focus, and quickly fusing to form longer rods and needles. The process shows most beautifully perhaps with solutions of fibrinogen and thrombin, but when the formation of crystals occurs quickly it is difficult to follow the process in detail. I have examined the solutions of thrombin, fibrinogen, and plasma, before mixing, and also mixtures made with varying proportions of thrombin. The aqueous solutions of thrombin employed, when examined under the ultramicroscope, showed scattered particles that exhibited Brownian movement as well as movement of translation through the field. The field itself was nearly dark showing only a faint indication of a cone even with the intense illumination of a bright sun. The solutions of fibrinogen and also the plasmas showed a number of particles in active Brownian movement, and in addition a strongly luminous cone in which individual particles could not be distinguished. According to my observations it is in this background of particles beyond the resolving power of the ultramicroscope that the needles are formed. In some cases (fibrinogen from terrapin's plasma) I have seen this uniform luminous field when thrombin was added, take on a granular or particulate appearance, the particles subsequently adhering in strings or threads as described by Mayer. When fibrinogen from the mammal was used and an adequate amount of thrombin, the bright bacillus-like rods that first appear are born in this field, and when the process is over and the meshwork of needles is formed the luminous background has disappeared. It would seem therefore that under the influence of the thrombin there is first an aggregation of the

[5] I am greatly indebted to Dr. Daniel Davis, assistant in physiology, for making the photographs used to illustrate this paper.

invisible particles (amicrons) to visible particles and then the further consolidation of these particles into the rigid looking needles. The active jumping movements of the short rods when first formed especially in the fibrinogen solutions, before they fuse into the longer needles, strongly suggest an influence of electrical attraction and repulsion. When the quantity of thrombin added is not sufficient to cause prompt clotting, the process of production of the needles is less striking. Individual needles are formed that float into and out of the cone of light, and often they appear slightly bent or curved. Eventually in such cases they form loose clusters or a loose meshwork, between which are seen many dancing particles. So far as my examination has gone all the plasmas used exhibit this needle formation, or deposition of fibrin crystals, but in the plasma of the horse and of the terrapin the needles are smaller and finer than in the plasmas of the dog or cat or in human plasma. So also in the fibrinogen made from the horse's plasma the needle formation is less striking than in that from the other mammals used (see fig. 5), while in the fibrinogen prepared from the terrapin's plasma the formation of the acicular crystals is still less complete. At the end of the process one sees a meshwork of threads or rods of varying length and size, exhibiting a beaded appearance as though composed of particles incompletely fused.

CONDITIONS MODIFYING THE FORMATION OF THE FIBRIN NEEDLES

Changes in the formation of the needles have been observed under the following conditions. (1) In cat's plasma made to clot by the addition of thrombin the size of the crystals varied with the rapidity of clotting. A large amount of thrombin caused very rapid clotting with the production of small although very distinct needles, while with a small amount of thrombin the clotting was slow and the needles were relatively very large, as seen in figure 4. (2) With very dilute solutions of fibrinogen and of thrombin such as give a loose clot, the fibrin needles were well formed but adhered in loose more or less separate clumps instead of forming a solid uniform meshwork. (3) In mixtures of fibrinogen and thrombin in which the amount of the latter

was insufficient to give a firm clot it was observed in several cases that the fusion of the particles might show varying degrees of completeness. In some cases the particles aggregated in smaller or larger clumps or heaps in which the separate particles were distinctly visible. In such cases the smaller clumps showed Brownian movements, and the separate particles in the clumps might exhibit independent movements. On standing the clumps may again disintegrate into particles or aggregations of particles. In other cases in which the thrombin was insufficient in amount the particles adhered to form short rods or longer beaded threads, giving the appearance of an abortive attempt at the production of needles. (4) Partially denatured fibrinogen gives an imperfect formation of fibrin needles under the influence of thrombin. This effect was observed especially with specimens of dried plasma. The author has employed dried plasma in experiments upon the quantitative estimation of antithrombin in the blood. This plasma is prepared by oxalating blood, centrifugalizing to get a clear plasma and then dializing against salt solution in tubes of collodion to remove the excess of oxalate. The plasma thus prepared may be dried in small quantities and used subsequently for coagulation experiments after redissolving in solutions of sodium chloride 0.9 per cent. These solutions give a good clot when thrombin is added, but under the ultramicroscope it was found that there was never a formation of typical fibrin needles. The meshwork was composed of short rods or strings or longer threads in which for the most part the separate particles could be distinguished.

SUMMARY

The paper gives a description of the phenomena observed under the ultramicroscope when solutions of thrombin are added to oxalated blood-plasma or to solutions of fibrinogen. The essential point is that the fibrin formed is deposited not as a network but as separate well-formed needles of crystalline appearance which are massed to produce a mesh. Under certain conditions the process is incomplete, giving rise to the production of threads or rods of different lengths in place of the needles.

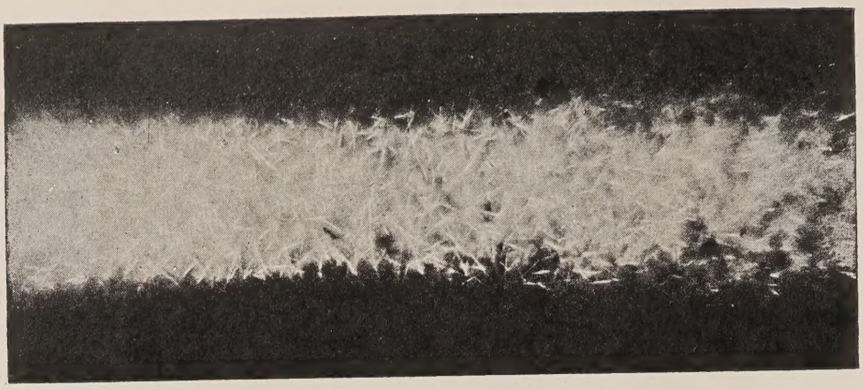

FIG. 1

FIG. 2

FIG. 3

Figs. 1, 2, and 3. Figure 1, oxalated plasma of human blood clotted with thrombin. Photographed under low magnification (257) to show a portion of the cone and the nature of the mesh formed by the fibrin needles. Figure 2, oxalated plasma of human blood clotted with thrombin, magnification 512, to show the details of the needles. Figure 3, Fibrinogen solution made from cat's plasma and clotted with thrombin, magnification 512.

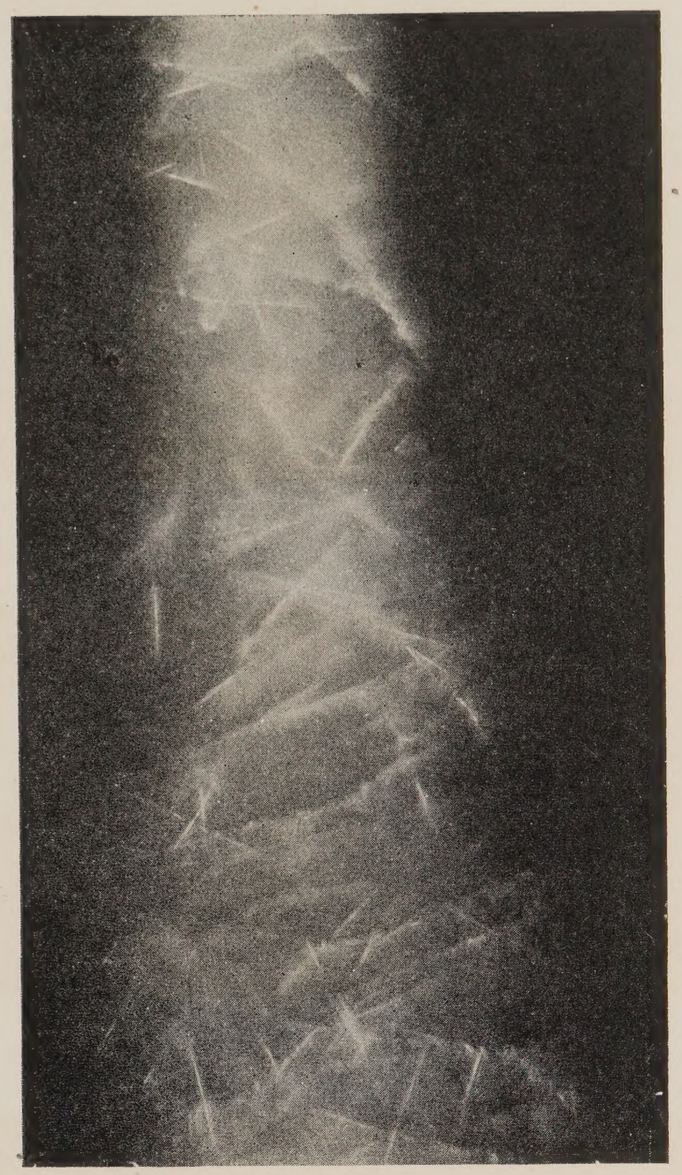

FIG. 4

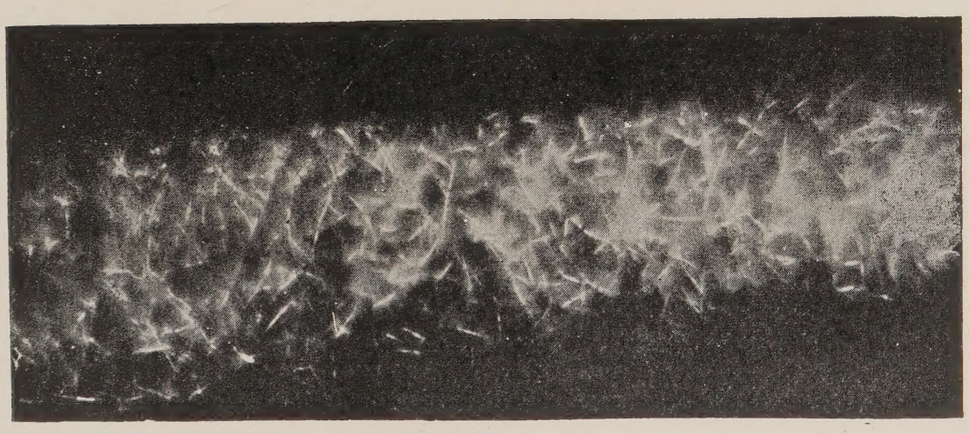

FIG. 5

Figs. 4 and 5. Figure 4, oxalated plasma of cat's blood clotted with thrombin. Magnification 512. To show the large needles obtained in slow clotting Figure 5, fibrinogen solution made from horse's plasma and clotted with thrombin. Magnification 512. To show less complete formation of needles.